Signaling Molecules and Pulp Regeneration

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Abstract

Signaling molecules play an essential role in tissue engineering because they regulate regenerative processes. Evidence exists from animal studies that single molecules such as members of the transforming growth factor beta superfamily and factors that induce the growth of blood vessels (vascular endothelial growth factor), nerves (brain-derived neurotrophic factor), or fibroblasts (fibroblast growth factor) may induce reparative dentin formation. Mainly the formation of atubular dentin (osteodentin) has been described after the application of single molecules or combinations of recombinant growth factors on healthy exposed pulps or in pulp regeneration. Generally, such preparations have not received regulatory approval on the market so far. Only the use of granulocyte colony-stimulating factors together with cell transplantation is presently tested clinically. Besides approaches with only 1 or few combined molecules, the exploitation of tissue-derived growth factors depicts a third promising way in dental pulp tissue engineering. Preparations such as platelet-rich plasma or platelet-rich fibrin provide a multitude of endogenous signaling molecules, and special regulatory approval for the market does not seem necessary. Furthermore, dentin is a perfect reservoir of signaling molecules that can be mobilized by treatment with demineralizing agents such as EDTA. This conditions the dentin surface and allows for contact differentiation of pulp stem cells into odontoblastlike cells, protects dentin from resorption, and enhances cell growth as well as attachment to dentin. By ultrasonic activation, signaling molecules can be further released from EDTA pretreated dentin into saline, thus avoiding cytotoxic EDTA in the final preparation. The use of dentin-derived growth factors offers a number of advantages because they are locally available and presumably are most fit to induce signaling processes in dental pulp. However, better characterization and standardization of the procedures are required. *(J Endod 2017;43:S7–S11)*

Key Words

Dental pulp tissue engineering, dentin matrix proteins, growth factors, pulp regeneration, scaffold

Significance

Signaling molecules are an essential element of tissue engineering because they regulate new tissue formation by controlling proliferation and differentiation. In this context, single recombinant proteins or mixtures of those have successfully been used to induce tertiary dentin formation and also for approaches to regenerate dental pulp. A clinically rather promising approach is the use of endogenous signaling molecules derived from local tissues, especially dentin, after conditioning with EDTA.

Langer and Vacanti (1) first outlined the basic prerequisites for tissue engineering in 1993. Accordingly, appropriate cells (stem or progenitor cells), a matrix that allows for cell growth, and appropriate signaling molecules for regulating the cellular processes are needed for new tissue formation. This concept can be applied to dental pulp regeneration (2). In this context, a number of signaling molecules from dentin matrix, as summarized in Table 1, have been described to have beneficial effects on chemotaxis/cell homing (eg, interleukin 8 and transforming growth factor beta 1 [TGF-β1]), angiogenesis (eg, vascular endothelial growth factor [VEGF]), neural growth (eg, brain-derived neurotrophic factor and glial cell line–derived neurotrophic factor), proliferation (eg, fibroblast growth factor 2 [FGF-2]), and differentiation (eg, TGF-β1) (3, 6–8).

Generally, such molecules can be obtained as recombinant proteins from commercial sources, from resident or transplanted cells, or in endogenous form from blood or local hard tissues. In case of dental pulp regeneration, they can be released from cells in the remaining pulpal and periapical tissues, mobilized from adjacent dentin, or introduced by a blood clot scaffold or platelet concentrates such as platelet-rich plasma and platelet-rich fibrin.

Single Signaling Molecules

Rutherford et al (9) exposed molar and premolar pulps in primates and capped the tissue with recombinant human osteogenic protein-1 (hOP-1, also referred to as BMP-7) in a matrix of bovine type-1 collagen. After 6 weeks, reparative dentin had formed in all treated teeth in the test group with an intact coronal seal (12/15). Less favorable results were obtained in teeth in which pulps were capped with a calcium hydroxide ($Ca[OH]_2$) paste. The collagen carrier alone did not induce dentin formation. Interestingly, the new dentin that formed after exposure to the signaling molecules seemed to replace the material that had been inserted, whereas after $Ca(OH)_2$ application new dentin was formed on the expense of the remaining pulp.

In 1994, Nakashima (10) reported on reparative dentin formation after the application of recombinant human BMP-2 and -4 together with a mixture of inactivated dentin matrix powder, chondroitin 6-sulfate sodium salt, and type I collagen in canine pulps after a pulpotomy procedure without any apparent adverse effect like inflammation. Two months after treatment, tubular dentin was observed in the lower part of the

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TABLE 1. Bioactive Components Available in Dentin Matrix (3–5)

Dentin-derived signaling molecules
Growth factors and cytokines
Bone morphogenetic protein 2, 4, and 7
Brain-derived neurotrophic factor
Epidermal growth factor
Fibroblast growth factor
Glial cell line-derived neurotrophic factor
Hepatocyte growth factor
Insulinlike growth factor 1 and 2
Nerve growth factor
Placenta growth factor
Platelet-derived growth factor
Transforming growth factor β1, -β2, and -β3
Vascular endothelial growth factor
Siblings
Bone sialoprotein
Dentin matrix protein 1
Dentin phosphoprotein
Dentin sialoprotein
Matrix extracellular phosphoglycoprotein
Osteopontin
Other
Adrenomedullin
Immunoglobulin G, A, and M
Interleukin 8 and 10

amputation cavity, whereas mainly atubular tertiary dentin with entrapped cells was produced in the upper part (osteodentin). In contrast to tubular dentin (orthodentin), osteodentin is characterized by a mainly atubular matrix with encapsulated cell bodies (11, 12), resembling osteocytes entrapped in bone tissue (13). Dentin formation was considerably less without signaling molecules (10). It is known that BMP-2 and -4 induce expression of the transcription factors msh homeobox 1 and 2 and are responsible for cell differentiation (14). The authors concluded that osteodentin formation as observed in this study was most likely caused by the recombinant proteins applied, whereas tubular dentin formation was ascribed to the dentin matrix (10). A review on dentinogenic properties of recombinant BMP-2, -4, and -7 by Rutherford et al in 1995 stated that these molecules are apparently able to induce reparative dentin formation in exposed healthy pulps of experimental animals (15).

The effect of recombinant BMP-7 (human osteogenic protein-1) in a collagen carrier on inflamed pulps was further studied by Rutherford et al in 2000 (16). The mixture was applied on exposed pulps in immature ferret teeth after previous treatment with lipopolysaccharides from *Salmonella typhimurium* for 3 days, which stimulated reversible pulpitis. After up to 2 months, it was obvious that the additional application of lipopolysaccharides blocked reparative dentin formation. Although the mechanism of this interaction is unclear, the results are in line with the clinical experience that bacteria and pulp inflammation negatively influence the success of pulp capping (14, 17).

Different recombinant human signaling molecules embedded in collagen gels were investigated by Suzuki et al (18), both *in vitro* on dental stem cells as well as *in vivo*. In the latter experiments, collagen gels with or without basic FGF were inserted into root canals of endodontically treated human teeth, which were subsequently implanted subcutaneously in rats. According to the authors, a basic FGF–adsorbed collagen scaffold led to recellularization of the root canals after 3 weeks *in vivo* (18). More recently, amelogenin was applied in a propylene glycol alginate vehicle in dogs to induce apical closure and pulp regeneration in open-apex, nonvital permanent canine teeth (19). The recombinant amelogenin protein had enhanced apex formation and promoted soft connective tissue regeneration after 6 months (19). Granulocyte colony-stimulating factor (G-CSF) and pulp-derived cells in a collagen carrier have been used for pulp regeneration in pulpectomized canine teeth. Pulp tissue was fully regenerated 90 days after the transplantation of pulp stem cells with G-CSF, which was detected histologically and confirmed by magnetic resonance imaging showing a signal intensity comparable with normal pulp tissue (20). It was further reported that G-CSF together with conditioned medium of pulp stem cells promoted immunosuppression *in vitro* (21).

In summary, the literature reports such as those mentioned previously have shown that single signaling molecules in different matrices can successfully be used for the treatment of exposed or amputated healthy pulps in experimental animals to induce reparative dentin formation and also for pulp regeneration approaches, as highlighted more recently. The use of single molecules seems to be a comparatively simple approach. The system is well-defined, and sufficient evidence from animal experimentation is available. It can be assumed that the use of 1 signaling molecule acts as a kickoff to start more complex signaling cascades that are necessary for new tissue formation because this process involves a large number of signaling molecules. However, information on optimal concentrations is purely empirical, and the costs for a treatment following this strategy are higher than for conventional methods. Potentially adverse effects have to be considered, such as an increased cancer risk or problems of immunogenicity (22, 23). Another problem might be the stability of these molecules in inflamed tissues. Furthermore, the question arises regarding which of the proposed signal molecules is best to choose. So far, none of the described molecules have been approved for clinical use in dentin/pulp regeneration although sufficient evidence exists from animal experiments that single signaling molecules induce reparative dentin formation. For pulp regeneration, single signaling molecules in combination with cell transplants are presently investigated in clinical trials (24).

Groups of Signaling Molecules

Signaling molecules (eg, VEGF, TGF-β1, and FGF-2) have been applied together with pulp-derived cells in human tooth roots and implanted subcutaneously in immunosuppressed mice (25). After 6 weeks, new tissue formation in the root canal was shown along with the formation of odontoblastlike cells at the interface to dentin (Fig. 1*A–D*). Similarly, many other growth factors and combinations have been tested for beneficial effects on pulp regeneration *in vivo* and *in vitro* (26, 27).

In summary, defined groups of signal molecules have successfully been used for dentin pulp regeneration procedures in experimental animals. In analogy to the use of single molecules, this approach is comparatively simple and well-defined. It seems to closer mimic the *in vivo* situation in which the interplay and potentially synergistic effects of different signaling factors are needed for tissue regeneration. On the other hand, even a group of signaling molecules does not reflect the complex interplay observed, for example, in wound healing (28, 29). Furthermore, open questions remain regarding the choice of signaling molecules and respective concentrations, the high cost, and the stability of the bioactive molecules. In conclusion, groups of molecules can be used together with cell transplants, but none of these approaches have obtained regulatory approval on the market for use in dental pulp regeneration.

Tissue-derived Signaling Molecules

As mentioned previously, mixtures of signaling molecules can be derived from the remaining parts of the pulp, from transplanted cells, from blood influx or inserted platelet concentrates, and at best from adjacent dentin. Dentin contains a large number of signaling molecules (3-5), which seem to play important roles in tissue repair and